1: Immunol Invest. 2002 Aug-Nov;31(3-4):247-62.

Links

Cross reaction of tetanus and botulinum neurotoxins A and B and the boosting effect of botulinum neurotoxins A and B on a primary anti-tetanus antibody response.

Dolimbek BZ, **Jankovic J**, **Atassi MZ**.

Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas, USA.

The present studies were carried out in order to investigate the cross-reaction of botulinum neurotoxins (BoNTs) with human and mouse antibodies against tetanus neurotoxin (TeNT) and determine whether injection of BoNT into a host that has been primed with TeNT would result in boosting of the response to the injected BoNT. Human antisera against TeNT obtained from 9 individuals were found to exhibit substantial cross-reaction with BoNTs A and B. We prepared antibodies (Abs) against inactivated tetanus neurotoxin (TeNT) in outbred mice and determined the binding of these Abs to active TeNT and active botulinum neurotoxins (BoNTs) A and B. Blood samples were collected before immunization (day 0) and on days 42, 82 and 125 after the first injection. The reactions of these sera with the immunizing antigen (inactivated TeNT), active TeNT, active BoNT/A and active BoNT/B were determined. At a fixed dilution (1:62.5 v/v), the sera contained high levels of Abs that reacted with TeNT and also with BoNTs A and B. Throughout the test period (up through day 125) and at different dilutions the cross-reactions of the antisera with BoNT/B were almost twice those with BoNT/A. The reactions of the antisera with the immunizing antigen (inactive TeNT) or with active TeNT were essentially equal throughout the dilution range tested (1:16-1:500 v/v). To determine whether injection of BoNT/A or B into a host that had been primed with TeNT resulted in boosting of the response to the priming antigen (TeNT) as well as BoNT/A or B, mice were primed with TeNT and boosted 21 days later with TeNT, BoNT/A or BoNT/B. Appropriate controls were also employed. Blood samples were collected prior to TeNT priming (day -1) and on days 21, 32, 46 and 67 after priming. In TeNT-primed mice, BoNTs A or B boosted the anti-TeNT Ab responses slightly but had no significant boosting effect on the Ab populations that bind to BoNTs A or B. It is concluded that while Abs against TeNT cross react with BoNTs and the cross reaction with BoNT/B is almost double that of BoNT/A, injection of BoNTs A or B in the presence of a prior active immunity against TeNT is not very likely to make the host mount an Ab response against the injected BoNT.

PMID: 12472183 [PubMed - indexed for MEDLINE]

1: Protein J. 2004 Jan;23(1):39-52.

Mapping of the antibody-binding regions on the HN-domain (residues 449-859) of botulinum neurotoxin A with antitoxin antibodies from four host species. Full profile of the continuous antigenic regions of the H-chain of botulinum neurotoxin A.

Atassi MZ, Dolimbek BZ.

Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas, USA. matassi@bcm.tmc.edu

Previously, we mapped the antibody (Ab) and T-cell recognition regions on the HC domain (residues 855-1296) of the 848-residue heavy (H) chain of botulinum neurotoxin A (BoNT/A). We have mapped here the HN-domain (residues 449-859) regions that bind protective anti-BoNT/A Abs raised in four different species. We synthesized, purified, and characterized 29 19-residue peptides that spanned the entire HN and overlapped consecutively by 5 residues, and also region L218-231 around the L-chain's substrate-binding site. Human, horse, mouse, and chicken anti-BoNT/A Abs did not bind to the L-peptide but recognized similar HN regions within peptides 519-537/533-551/547-565/561-579 (with slight left- or right-shifts), 743-761, 785-803, and 813-831/827-845 overlap. Recognition of other peptides that bound lower Ab levels showed similarities and also some differences. Peptide 463-481, strongly immunodominant with horse antisera, did not bind human, mouse, and chicken Abs. However, peptide 449-467 bound Abs in these three antisera, and the region may have shifted to the right (peptide 463-481) with horse Abs. The overlap 659-677/673-691 reacted strongly with human Abs whereas with mouse and chicken antisera, only peptide 673-691 showed low reactivity. Horse antisera had no detectable Ab binding to region(s) 659-691. The Ab-recognition regions on the H chain occupy surface locations in BoNT/A three-dimensional structure, but the great part of the surface is not immunogenic. Regions recognized by the protective antisera of the four different species are prime candidates for inclusion in synthetic vaccine designs.

PMID: 15115181 [PubMed - indexed for MEDLINE]

Oct 24 2006 07:33:51

P04958 TETX_CLOTE **Tetanus toxin precursor (EC 3.4.24.68) (Tentoxylysin) [Contains: Tetanus toxin light chain (Tetanus toxin chain L); Tetanus toxin heavy chain (Tetanus toxin chain H)] [tetX] [Clostridium tetani]**

Score = 1638 bits (4242), Expect = 0.0
Identities = 823/864 (95%), Positives = 823/864 (95%)

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Query: 1    LYNRTASLTDLGGELCIKIKNEDLTFIAEKNSFSEEPFQDEIVSYNTKNKPLNFN
            LYNRTASLTDLGGELCIKIKNEDLTFIAEKNSFSEEPFQDEIVSYNTKNKPLNFN
Sbjct: 451  LYNRTASLTDLGGELCIKIKNEDLTFIAEKNSFSEEPFQDEIVSYNTKNKPLNFN

Query: 61   IIVDYNLQSKITLPNDRTTPVTKGIPYAPEYKSNAASTIEIHNIDDNTIYQYLYA
            IIVDYNLQSKITLPNDRTTPVTKGIPYAPEYKSNAASTIEIHNIDDNTIYQYLYA
Sbjct: 511  IIVDYNLQSKITLPNDRTTPVTKGIPYAPEYKSNAASTIEIHNIDDNTIYQYLYA

Query: 121  TLQRITMTNSVDDALINSTKIYSYFPSVISKVNQGAQGILFLQWVRDIIDDFTNE
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Sbjct: 571  TLQRITMTNSVDDALINSTKIYSYFPSVISKVNQGAQGILFLQWVRDIIDDFTNE

Query: 181  TIDKISDVSTIVPYIGPALNIVKQGYEGNFIGALETTGVVLLLEYIPEITLPVIA
            TIDKISDVSTIVPYIGPALNIVKQGYEGNFIGALETTGVVLLLEYIPEITLPVIA
Sbjct: 631  TIDKISDVSTIVPYIGPALNIVKQGYEGNFIGALETTGVVLLLEYIPEITLPVIA

Query: 241  ESSTQKEKIIKTIDNFLEKRYEKWIEVYKLVKAKWLGTVNTQFQKRSYQMYRSLE
            ESSTQKEKIIKTIDNFLEKRYEKWIEVYKLVKAKWLGTVNTQFQKRSYQMYRSLE
Sbjct: 691  ESSTQKEKIIKTIDNFLEKRYEKWIEVYKLVKAKWLGTVNTQFQKRSYQMYRSLE

Query: 301  XXXXXXXXXXXXSGPDKEQIADEIXXXXXXXXXXXXXXMININIFMRESSRSFLV
                        SGPDKEQIADEI              MININIFMRESSRSFLV
Sbjct: 751  IKKIIDYEYKIYSGPDKEQIADEINNLKNKLEEKANKAMININIFMRESSRSFLV

Query: 361  EAKKQLLEFDTQSKNILMQYIKANSKFIGITELKKLESKINKVFSTPIPFSYSKN
            EAKKQLLEFDTQSKNILMQYIKANSKFIGITELKKLESKINKVFSTPIPFSYSKN
Sbjct: 811  EAKKQLLEFDTQSKNILMQYIKANSKFIGITELKKLESKINKVFSTPIPFSYSKN

Query: 421  DNEEDIDVILKKSTXXXXXXXXXXXXXXXGFNSSVITYPDAQLVPGINGKAIHLV
            DNEEDIDVILKKST               GFNSSVITYPDAQLVPGINGKAIHLV
Sbjct: 871  DNEEDIDVILKKSTILNLDINNDIISDISGFNSSVITYPDAQLVPGINGKAIHLV

Query: 481  EVIVHKAMDIEYNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISSMKKHSLS
            EVIVHKAMDIEYNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISSMKKHSLS
Sbjct: 931  EVIVHKAMDIEYNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISSMKKHSLS

Query: 541  SVSLKGNNLIWTLKDSAGEVRQITFRDLPDKFNAYLANKWVFITITNDRLSSANL
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Sbjct: 991  SVSLKGNNLIWTLKDSAGEVRQITFRDLPDKFNAYLANKWVFITITNDRLSSANL
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Query: 601  LMGSAEITGLGAIREDNNITLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYT
            LMGSAEITGLGAIREDNNITLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYT
Sbjct: 1051 LMGSAEITGLGAIREDNNITLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYT

Query: 661  TFLRDFWGNPLRYDTEYYLIPVASSSKDVQLKNITDYMYLTNAPSYTNGKLNIYY
            TFLRDFWGNPLRYDTEYYLIPVASSSKDVQLKNITDYMYLTNAPSYTNGKLNIYY
Sbjct: 1111 TFLRDFWGNPLRYDTEYYLIPVASSSKDVQLKNITDYMYLTNAPSYTNGKLNIYY

Query: 721  GLKFIIKRYTPNNEIDSFVKSGDFIKLYVSYNNNEHIVGYPKDGNAFNNLDRILR
            GLKFIIKRYTPNNEIDSFVKSGDFIKLYVSYNNNEHIVGYPKDGNAFNNLDRILR
Sbjct: 1171 GLKFIIKRYTPNNEIDSFVKSGDFIKLYVSYNNNEHIVGYPKDGNAFNNLDRILR

Query: 781  PGIPLYKKMEAVKLRDLKTYSVQLKLYDDKNASLGLVGTHNGQIGNDPNRDILIA
            PGIPLYKKMEAVKLRDLKTYSVQLKLYDDKNASLGLVGTHNGQIGNDPNRDILIA
Sbjct: 1231 PGIPLYKKMEAVKLRDLKTYSVQLKLYDDKNASLGLVGTHNGQIGNDPNRDILIA

Query: 841  NHLKDKILGCDWYFVPTDEGWTND 864
            NHLKDKILGCDWYFVPTDEGWTND
Sbjct: 1291 NHLKDKILGCDWYFVPTDEGWTND 1314
```

Protein J. 2004 Nov;23(8):539-52.

Links

Mapping of the synaptosome-binding regions on the heavy chain of botulinum neurotoxin A by synthetic overlapping peptides encompassing the entire chain.

Maruta T, **Dolimbek BZ**, **Aoki KR**, **Steward LE**, **Atassi MZ**.

Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas 77030, USA.

The purpose of this work was to map, on the heavy (H) chain of botulinum neurotoxin A (BoNT/A), the regions that bind to mouse brain synaptosomes (snps). We prepared 60 synthetic overlapping peptides that had uniform size and overlaps and encompassed the entire H chain (residues 499 to 1296) of BoNT/A. The ability of each peptide to inhibit the binding of 125I-labeled BoNT/A to mouse brain snps was studied. The binding of 125I-labeled BoNT/A to mouse brain snps was completely inhibited by free unlabeled BoNT/A, but not by unrelated proteins, indicating that the binding of BoNT/A to mouse brain snps was a specific event. Inhibition studies with the individual peptides showed that, on the H(N) domain, inhibitory activities greater than 10% were exhibited, in decreasing order, by peptides 799-817, 659-677, 729-747, 533-551, 701-719, and 757-775. Lower inhibitory activities (between 5.6% and 8.7%) were exhibited by five other peptides, 463-481, 505-523, 519-537, 603-621 and 645-663. The remaining 18 H(N) peptides had little or no inhibitory activity. In the H(C) domain, peptides 1065-1083, 1163-1181 and 1275-1296 had the highest inhibitory activities (between 25% and 29%), followed (10-12% inhibitory activity) by peptides 1107-1125, 1191-1209 and 1233-1251. Two other peptides, 1079-1097 and 1177-1195, had very low (5.8% and 4.9%) inhibitory activities. The remaining 23 H(C) peptides had no inhibitory activity. Inhibition with mixtures of equimolar quantities of the most active 6 peptides of HN, 5 of H(C) or all 11 of H(N) and H(C) revealed that the peptides contain independent non-competing binding regions. Comparison of the locations of the snp-binding regions on the H-subunit with the regions that bind blocking mouse anti-BoNT/A Abs helped explain the protecting ability of these Abs. In the three-dimensional structure of BoNT/A, the snp-binding regions that completely coincide or significantly overlap with the antigenic regions occupy surface locations and most of them reside in the last half of the H(C) domain. But some of the regions reside in the HN domain and might play a role in the translocation event.

PMID: 15648976 [PubMed - indexed for MEDLINE]

```
CLUSTAL FORMAT for T-COFFEE Version_1.37, CPU=0.00 sec, SCORE=16750, Nseq=2, Len=134

unk|VIRT3669|Blast_submission  -PITINNFRYSDPVNNDTIIMMEPPYCKGLDIYYKAFKITDRIWIVPERYEFG
tr|Q7B8V4|Q7B8V4_CLOBO         MPFVNKQFNYKDPVNGVDIAYIKIPNA-GQMQPVKAFKIHNKIWVIPERDTFT
                                *:. ::*.*.****.  *  :: * . *     ***** ::**::***  *

unk|VIRT3669|Blast_submission  NPPSSLIEGASEYYDPNYLRTDSDKDRFLQTMVKLFNRIKNNVAGEALLDKII
tr|Q7B8V4|Q7B8V4_CLOBO         NPPPEAKQVPVSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIV
                               ***..  : . .***..** **.:**.:*: :.***:** ..  *. ** .*:

unk|VIRT3669|Blast_submission  NSYSLLDKFDTNSNSVSFNLLEQDPSGATTKSAMLTNLIIFGPGPVLNKNEVR
tr|Q7B8V4|Q7B8V4_CLOBO         GS-----TIDTELKVIDTNCINVIQPDGSYRSEEL-NLVIIGPSADIIQFECK
                               .*     .:**: : :. * ::   ...: :*  * **:*:**.. : : * :

unk|VIRT3669|Blast_submission  NKNYFPCRDGFGSIMQMAFCPEYVPTFDNVIENITSLTIGKSKYFQDPALLLM
tr|Q7B8V4|Q7B8V4_CLOBO         NLT----RNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLA
                               * .    *:*:**   : *.*::.  *:: :*  *.  :* .*:  ***: *

unk|VIRT3669|Blast_submission  HGLYGMQVSSHEIIPSKQEIYMQHT-YPISAEELFTFGGQDANLISIDIKNDL
tr|Q7B8V4|Q7B8V4_CLOBO         HRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEF
                               * ***: :..:.::  : : * : :   :* *** ****:**::*.   :*::

unk|VIRT3669|Blast_submission  YKAIANKLSQVTSCNDPNIDIDSYKQIYQQKYQFDKDSNGQYIVNEDKFQILY
tr|Q7B8V4|Q7B8V4_CLOBO         FKDIASTLNKAKSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLY
                               :* **..*.:..*  ... .::  *::::::** :.:*:.*:: *:: **: **

unk|VIRT3669|Blast_submission  TEIELGKKFNIKTRLSYFSMNHDPVKIPNLLDDTIYNDTEGFNIESKDLKSEY
tr|Q7B8V4|Q7B8V4_CLOBO         TEDNFVKFFKVLNRKTYLNFDKAVFKI-NIVPKVNYTIYDGFNLRNTNLAANF
                               ** :: * *:: .* :*:.:::  .** *:: .. *.  :***:...:* :::

unk|VIRT3669|Blast_submission  NTNAF---RNVDGSGLVSKLIGLCKKIIPPTNIRENLYNRTASLTDLGGELCI
tr|Q7B8V4|Q7B8V4_CLOBO         NNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKALN------DLCI
                               *.  *   :*. *   . **: :   * . *:  :: **:: .      :***

unk|VIRT3669|Blast_submission  TFIAEKNSFSEEPFQDEIVSYNTKNKPLNFNYSLDKIIVDY------NLQSKI
tr|Q7B8V4|Q7B8V4_CLOBO         FFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENI
                                * ..:::.*:::  :.* :: :*: :. : * *** *   *      *  .:*

unk|VIRT3669|Blast_submission  TPVTKGIPYAPEYKSNAASTIEIHNIDDNTIYQYLYAQKSPTTLQRITMTNSV
tr|Q7B8V4|Q7B8V4_CLOBO         SDIIGQLELMPNIERFPNG--KKYELDKYTMFHYLRAQEFEHGKSRIALTNSV
                               : :   :   *: :  . .  : :::*. *:::** **:     .**::****

unk|VIRT3669|Blast_submission  TKIYSYFPS-VISKVNQGAQGILFLQWVRDIIDDFTNESSQKTTIDKISDVST
tr|Q7B8V4|Q7B8V4_CLOBO         SRVYTFFSSDYVKKVNKATEAAMFLGWVEQLVYDFTDETSEVSTTDKIADITI
                               :::*::*.*  :.***:.::. :** **.::: ***:*:*: :* ***:*::

unk|VIRT3669|Blast_submission  ALNIVKQGYEGNFIGALETTGVVLLLEYIPEITLPVIAALSIAESSTQKEKII
tr|Q7B8V4|Q7B8V4_CLOBO         ALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALVSYIANKVLTV
                               **** :  *:.:*:***  :*.*:***:****::**:.::::..  ::*   :

unk|VIRT3669|Blast_submission  EKRYEKWIEVYKLVKAKWLGTVNTQFQKRSYQMYRSLEYQVDAIKKIIDYEYK
tr|Q7B8V4|Q7B8V4_CLOBO         SKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQYN
                               .** *** **** : ::**..****::    :* .:** *.:* * **:*:*:

unk|VIRT3669|Blast_submission  EQIADEINNLKNKLEEKANKAMININIFMRESSRSFLVNQMINEAKKQLLEFD
tr|Q7B8V4|Q7B8V4_CLOBO         NNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFD
                               ::*  :*::*..**:*. ******** *:.:.* *:*:*.**  . *:* :**

unk|VIRT3669|Blast_submission  MQYIKANSKFIGITELKKLESKINKVFSTPIPFSYSKNLDCWVDNEEDIDVIL
tr|Q7B8V4|Q7B8V4_CLOBO         LKYIYDNRGTL-IGQVDRLKDKVNNTLSTDIPFQLSK----YVDNQRLLSTFT
```

sp P10845 BXA1_CLOBO **Botulinum neurotoxin type A precursor (EC 3.4.24.69) (BoNT/A) (Bontoxilysin-A) (BOTOX) [Contains: Botulinum neurotoxin A light-chain; Botulinum neurotoxin A heavy-chain] [botA] [Clostridium botulinum]**

Score = 645 bits (1665), Expect = 0.0
Identities = 438/1339 (32%), Positives = 683/1339 (51%), Gaps = 77

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Query: 1    PITINNFRYSDPVNNDTIIMMEPPYCKGLDIYYKAFKITDRIWIVPERYEFGTKP
            P     F Y DPVN   I  ++ P    +    KAFKI ++IW++PER  F T P
Sbjct: 1    PFVNKQFNYKDPVNGVDIAYIKIPNVGQMQPV-KAFKIHNKIWVIPERDTF-TNP

Query: 59   NPPSSLIEGASEYYDPNYLRTDSDKDRFLQTMVKLFNRIKNNVAGEALLDKIINA
            NPP    +    YYD  YL TD++KD +L+ + KLF RI +   G  LL  I+
Sbjct: 59   NPPPEAKQVPVSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRG

Query: 119  NSY--SLLDKFDTNSNSVSFNLLEQDPSGATTKSAMLTNLIIFGPGPVLNKNEVR
             S   + L   DTN      N+++ D S  + +     NL+I GP  + + E +
Sbjct: 119  GSTIDTELKVIDTNC----INVIQPDGSYRSEE----LNLVIIGPSADIIQFECK

Query: 177  VDNKNYFPCRDGFGSIMQMAFCPEYVPTFDNVIENITSLTIGKSKYFQDPALLLM
            V N      R+G+GS   + F P++   F+  +E  T+  +G  K+  DPA+ L
Sbjct: 171  VLNLT----RNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLA

Query: 237  VLHGLYGMQVSSHEIIPSKQEIYMQHT-YPISAEELFTFGGQDANLISIDIKNDL
              H LYG+ ++ + +       Y + +   +S EEL TFGG DA  I    +N+
Sbjct: 227  AGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEF

Query: 296  NDYKAIANKLSQVTSCNDPNIDIDSYKQIYQQKYQFDKDSNGQYIVNEDKFQILY
            N +K IA+ L++  S       +   K ++++KY   +D++G++ V++ KF  LY
Sbjct: 287  NKFKDIASTLNKAKSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLY

Query: 356  GFTEIELGKKFNIKTRLSYFSMNHDPVKIPNLLDDTIYNDTEGFNIESKDLKSEY
             +TE    K F +  R +Y + +    KI N++    Y   +GFN+ + +L + +
Sbjct: 347  IYTEDNFVKFFKVLNRKTYLNFDKAVFKI-NIVPKVNYTIYDGFNLRNTNLAANF

Query: 416  RVNTNAF---RNVDGSGLVSKLIGLCKKIIPPTNIRENLYNRTASLTDLGGELCI
             +N   F   +N  G     KL+ +   I   T   + YN+  +       +LCI
Sbjct: 406  EINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKALN------DLCI

Query: 473  DLTFIAEKNSFSEEPFQDEIVSYNTKNKPLNFNYSLDKIIVDY------NLQSKI
            DL F   +++F+ +  + E ++ +T  +    N SLD I   Y      N    I
Sbjct: 460  DLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENI

Query: 527  RTTPVTKGIPYAPEYKSNAASTIEIHNIDDNTIYQYLYAQKSPTTLQRITMTNSV
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            ++ +   +   P  +        + + +D  T++ YL AQ+      RI +TNSV
Sbjct: 519  LSSDIIGQLELMPNIERFPNG--KKYELDKYTMFHYLRAQEFEHGKSRIALTNSV

Query: 587  NSTKIYSYFPS-VISKVNQGAQGILFLQWVRDIIDDFTNESSQKTTIDKISDVST
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Sbjct: 577  NPSRVYTFFSSDYVKKVNKATEAAMFLGWVEQLVYDFTDETSEVSTTDKIADITI

Query: 646  GPALNIVKQGYEGNFIGALETTGVVLLLEYIPEITLPVIAALSIAESSTQKEKII
            GPALNI    Y+ +F+GAL  +G V+LLE+IPEI +PV+   ++       K  +
Sbjct: 637  GPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALVSYIANKVLTV

Query: 706  FLEKRYEKWIEVYKLVKAKWLGTVNTQFQKRSYQMYRSLEYQVDAXXXXXXXXXX
             L KR EKW EVYK +   WL  VNTQ      +M  +LE Q +A
Sbjct: 697  ALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQYN

Query: 766  DKEQIADEIXXXXXXXXXXXXXXMININIFMRESSRSFLVNQMINEAKKQLLEFD
            +K  I   I               MININ F+ + S S+L+N MI    K+L +FD
Sbjct: 757  EKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFD

Query: 826  ILMQYIKANSKFIGITELKKLESKINKVFSTPIPFSYSKNLDCWVDNEEDIDVIL
             L++YI  N   + I ++ +L+ K+N   ST IPF  SK    +VDN+  +
Sbjct: 817  ALLKYIYDNRGTL-IGQVDRLKDKVNNTLSTDIPFQLSK----YVDNQRLLSTFT

Query: 883  --STXXXXXXXXXXXXXXGFNSSVITYPDAQLVPGINGKAIHLVNNESSEVIVH
              +T                +S I          I+   I L N ESS++ V
Sbjct: 872  IINTSILNLRYESNHLIDLSRYASKINIGSKVNFDPIDKNQIQLFNLESSKIEVI

Query: 941  EYNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISSMKKHSLSIGSGWSVSLK
             YN M+ NF+ SFW+R+PK   S       NEY+II+ M+ +     SGW VSL
Sbjct: 932  VYNSMYENFSTSFWIRIPKYFNSISL---NNEYTIINCMENN-----SGWKVSLN

Query: 1001 WTLKDSAGEVRQITFRDLPDKFNAYLANKWVFITITNDRLSSANLYINGVLMGSA
            WTL+D+    +++ F+       +   N+W+F+TITN+RL+++ +YING L+
Sbjct: 984  WTLQDTQEIKQRVVFKYSQMINISDYINRWIFVTITNNRLNNSKIYINGRLIDQK

Query: 1061 GAIREDNNITLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLRD
            G I   NNI  KLD C + ++Y+ I  F +F K LN KEI+ LY +  +   L+D
Sbjct: 1044 GNIHASNNIMFKLDGCRDTHRYIWIKYFNLFDKELNEKEIKDLYDNQSNSGILKD

Query: 1121 LRYDTEYYLIPVASSSKDVQLKN--ITDYMYLTNAPSYTNGKLNIYYR-RLYNGL
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Sbjct: 1104 LQYDKPYYMLNLYDPNKYVDVNNVGIRGYMYL-KGPRGSVMTTNIYLNSSLYRGT

Query: 1178 RYTPNNEIDSFVKSGDFIKLYVSYNNNEHIVGYPKDGNAFN-NLDRILRVGYNAP
            +Y   N+ D+ V++ D + + V   N E    Y    NA    +++IL      P
Sbjct: 1163 KYASGNK-DNIVRNNDRVYINVVVKNKE----YRLATNASQAGVEKILS-ALEIP
```

```
Query: 1237 KKMEAVKLRDLK--TYSVQLKLYDDKNASLGLVGTHNGQIGNDPNRDILIASNWY
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Sbjct: 1217 SQVVVMKSKNDQGITNKCKMNLQDNNGNDIGFIGFH--QFNNIAK---LVASNWY

Query: 1295 --DKILGCDWYFVPTDEGW 1311
              + LGC W F+P D+GW
Sbjct: 1272 RSSRTLGCSWEFIPVDDGW 1290
```

```
                                ::**  *   : * ::.:*:.*:*:.:** ***. **    :***:. :..:

unk|VIRT3669|Blast_submission   KSTILNLDINNDIISDISGFNSSVITYPDAQLVPGINGKAIHLVNNESSEVIV
tr|Q7B8V4|Q7B8V4_CLOBO          NTSILNLRYESNHLIDLSRYASKINIGSKVNFDP-IDKNQIQLFNLESSKIEV
                                :::**** :.: : *:* : *.:   ...:: * *: : *:*.* ***:: *

unk|VIRT3669|Blast_submission   YNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISSMKKHSLSIGSGWSVSL
tr|Q7B8V4|Q7B8V4_CLOBO          YNSMYENFSTSFWIRIPKYFNSISL---NNEYTIINCMENN-----SGWKVSL
                                **.*::**:.***:*:**   *      .***:**..*:::     ***.***

unk|VIRT3669|Blast_submission   TLKDSAGEVRQITFRDLPDKFNAYLANKWVFITITNDRLSSANLYINGVLMGS
tr|Q7B8V4|Q7B8V4_CLOBO          TLQDTQEIKQRVVFKYSQMINISDYINRWIFVTITNNRLNNSKIYINGRLIDQ
                                **:*:    :::.*:       :   *:*:*:****:**..:::**** *:..

unk|VIRT3669|Blast_submission   AIREDNNITLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLR
tr|Q7B8V4|Q7B8V4_CLOBO          NIHASNNIMFKLDGCRDTHRYIWIKYFNLFDKELNEKEIKDLYDNQSNSGILK
                                 *: .*** :*** *.:.::*: *. *.:* * ** ***:.** .  .  :*:

unk|VIRT3669|Blast_submission   RYDTEYYLIPVASSSKDVQLKN--ITDYMYLTNAPSYTNGKLNIYYRR-LYNG
tr|Q7B8V4|Q7B8V4_CLOBO          QYDKPYYMLNLYDPNKYVDVNNVGIRGYMYL-KGPRGSVMTTNIYLNSSLYRG
                                :**. **:: : ...* *:::* * .**** :.*  :  . *** .  **.*

unk|VIRT3669|Blast_submission   YTPNNEIDSFVKSGDFIKLYVSYNNNEHIVGYPKDGNAFNNLDRILRVGYNAP
tr|Q7B8V4|Q7B8V4_CLOBO          YASGNK-DNIVRNNDRVYINVVVKNKEYRLATNASQAGVEKILSALEI----P
                                *:..*: *.:*:..* : : *  :*:*: :.   .  ..:::   *.:    *

unk|VIRT3669|Blast_submission   MEAVKLRDLK--TYSVQLKLYDDKNASLGLVGTHNGQIGNDPNRDILIASNWY
tr|Q7B8V4|Q7B8V4_CLOBO          VVVMKSKNDQGITNKCKMNLQDNNGNDIGFIGFHQF------NNIAKLVASNW
                                : .:* :: :  * . :::* *::. .:*::* *:       *.   :.:. :

unk|VIRT3669|Blast_submission   KDKILGCDWYFVPTDEGWTND--
tr|Q7B8V4|Q7B8V4_CLOBO          SSRTLGCSWEFIPVDDGWGERPL
                                :.: ***.* *:*.*:** :
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CLUSTAL FORMAT for T-COFFEE Version_1.37, CPU=0.00 sec, SCORE=3830, Nseq=2, Len=1337

unk|VIRT5247|Blast_submission  --------------------------------------------------
sp|P10845|BXA1_CLOBO           PFVNKQFNYKDPVNGVDIAYIKIPNVGQMQPVKAFKIHNKIWVIPERDTFTNP

unk|VIRT5247|Blast_submission  --------------------------------------------------
sp|P10845|BXA1_CLOBO           PPEAKQVPVSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRG

unk|VIRT5247|Blast_submission  --------------------------------------------------
sp|P10845|BXA1_CLOBO           TIDTELKVIDTNCINVIQPDGSYRSEELNLVIIGPSADIIQFECKSFGHEVLN

unk|VIRT5247|Blast_submission  --------------------------------------------------
sp|P10845|BXA1_CLOBO           STQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHELIHAGHRLYG

unk|VIRT5247|Blast_submission  --------------------------------------------------
sp|P10845|BXA1_CLOBO           VFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIA

unk|VIRT5247|Blast_submission  -LYNRTASLTDLGG-----------------------------------
sp|P10845|BXA1_CLOBO           SIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNF
                                : . ****  : .

unk|VIRT5247|Blast_submission  --------------------------------------------------
sp|P10845|BXA1_CLOBO           NRKTYLNFDKAVFKINIVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFT

unk|VIRT5247|Blast_submission  ------------------------------ELCIKIKNEDLTFIAEKNSFSEE
sp|P10845|BXA1_CLOBO           LFEFYKLLCVRGIITSKTKSLDKGYNKALNDLCIKVNNWDLFFSPSEDNFTND
                                                    :****::* ** * ..::.*:::

unk|VIRT5247|Blast_submission  SYNTKNKPLNFNYSLDKIIVDY------NLQSKITLPNDRTTPV--TKGIPYA
sp|P10845|BXA1_CLOBO           TSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELMPNI
                               : :*: :. : * *** *   *      *  .:*:: *  :  :   : :*

unk|VIRT5247|Blast_submission  ASTIEIHNIDDNTIYQYLYAQKSPTTLQRITMTNSVDDALINSTKIYSYFPSV
sp|P10845|BXA1_CLOBO           KYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDY----
                                  :: :.: .    * :   **  :* . .    :: : : :    .*

unk|VIRT5247|Blast_submission  AQGILFLQWVRDIIDDFTNESSQKTTIDKISDVSTIVPYIGPALNIVKQGYEG
sp|P10845|BXA1_CLOBO           TEAAMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKD
                               ::. :** **.::: ***:*:*: :* ***:*:: *:*********  :  *:.

unk|VIRT5247|Blast_submission  TTGVVLLLEYIPEITLPVIAALSIAESSTQKEKIIKTIDNFLEKRYEKWIEVY
sp|P10845|BXA1_CLOBO           FSGAVILLEFIPEIAIPVLGTFALVSYIANKVLTVQTIDNALSKRNEKWDEVY
                                :*.*:***:****::**:.:::::..  ::*   ::**** *.** *** ***

unk|VIRT5247|Blast_submission  LGTVNTQFQKRSYQMYRSLEYQVDAIKKIIDYEYKIYSGPDKEQIADEINNLK
sp|P10845|BXA1_CLOBO           LAKVNTQIDLIRKKMKEALENQAEATKAIINYQYNQYTEEEKNNINFNIDDLS
                               *..****::    :* .:** *.:* * **:*:*: *:  :*::*  :*::*.

unk|VIRT5247|Blast_submission  NKAMININIFMRESSRSFLVNQMINEAKKQLLEFDTQSKNILMQYIKANSKFI
sp|P10845|BXA1_CLOBO           NKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYDNRG--
                               ******** *:.:.* *:*:*.**  . *:* :**:. *: *::**  *

unk|VIRT5247|Blast_submission  LESKINKVFSTPIPFSYSKNLDCWVDNEEDIDVILKKSTILNLDINNDIISDI
sp|P10845|BXA1_CLOBO           VDRLKDKVNNT-----LSTDIPFQLSKYVDNQRLLSTFTEYIKNIINTSILNL
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                                ::   :** .*      *.::   :.:  * : :*.. *    :* *  * ::

unk|VIRT5247|Blast_submission   ITYPDAQLVPGINGKA---------IHLVNNESSEVIVHKAMDIEYNDMFNNF
sp|P10845|BXA1_CLOBO            IDLSRYASKINIGSKVNFDPIDKNQIQLFNLESSKIEVILKNAIVYNSMYENF
                                *  .      .*..*.         *:*.* ***:: *     * **.*::**

unk|VIRT5247|Blast_submission   VPKVSASHLEQYGTNEYSIISSMKKHSLSIGSGWSVSLKGNNLIWTLKDSAGE
sp|P10845|BXA1_CLOBO            IPKYFNSISL---NNEYTIINCMENN-----SGWKVSLNYGEIIWTLQDTQEI
                                :**   *      .***:**..*:::     ***.***: .::****:*:

unk|VIRT5247|Blast_submission   DLPDKFNAYLANKWVFITITNDRLSSANLYINGVLMGSAEITGLGAIREDNNI
sp|P10845|BXA1_CLOBO            YSQMINISDYINRWIFVTITNNRLNNSKIYINGRLIDQKPISNLGNIHASNNI
                                       :   *:*:*:****:**..:::**** *:.  *:.** *: .***

unk|VIRT5247|Blast_submission   NNNNQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLRDFWGNPLRYDTEYYL
sp|P10845|BXA1_CLOBO            RDTHRYIWIKYFNLFDKELNEKEIKDLYDNQSNSGILKDFWGDYLQYDKPYYM
                                .:.::*: *. *.:* * ** ***:.** .  .  :*:****: *:**. **:

unk|VIRT5247|Blast_submission   KDVQLKNITDYMYL-----TNAPSYTNGKLNIYYRRLYNGLKFIIKRYTPNNE
sp|P10845|BXA1_CLOBO            KYVDVNNVGIRGYMYLKGPRGSVMTTNIYLNSSLYRGTKFIIKKYASGNKDNI
                                * *:::*:    *:      .:   **  **    *  : :       . :*

unk|VIRT5247|Blast_submission   VKSGDFIKLYVSYNNNEHIVGYPKDGNAFNNLDRILRVGYNAPGIPLYKKMEA
sp|P10845|BXA1_CLOBO            YINVVVKNKEYRLATNASQAGVEKILSALEIPD----VGNLSQVVVMKSKND-
                                  .  . :      .*   .*  *  .*::  *    **  :  : : .* :

unk|VIRT5247|Blast_submission   TYSVQLKLYDDKNASLGLVGTHNGQ------IGNDPNRDI-----LIASNWYF
sp|P10845|BXA1_CLOBO            TNKCKMNLQDNNGNDIGFIGFHQFNNIAKLVASNWYNRQIERSSRTLGCSWEF
                                * . :::* *::. .:*::* *: :       .*  **:*      :...* *

unk|VIRT5247|Blast_submission   LGCDWYFVPTDEGWTND
sp|P10845|BXA1_CLOBO            ---GWGERPL-------
                                   .*   *
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1: Autoimmunity. 1998;27(2):79-90.

Links

T cell responses in EAMG-susceptible and non-susceptible mouse strains after immunization with overlapping peptides encompassing the extracellular part of Torpedo californica acetylcholine receptor alpha chain. Implication to role in myasthenia gravis of autoimmune T-cell responses against receptor degradation products.

Oshima M, Yokoi T, Deitiker P, Atassi MZ.

Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, TX 77030, USA.

To study the role in myasthenia gravis (MG) of peptides resulting from acetylcholine receptor (AChR) degradation, we examined the ability of AChR peptides to induce T cell responses that are capable of cross-reacting with intact AChR. The studies were carried out in an experimental autoimmune MG (EAMG)-susceptible mouse strain [C57BL/6 (B6)] as well as in two non-susceptible strains [B6.C-H-2bm12 (bm12) and C3H/He]. A set of overlapping peptides encompassing the extracellular part (residues 1-210) of the alpha-chain of Torpedo californica (t) AChR were used, individually or in equimolar mixtures, as immunogens. In B6, immunization with peptides alpha45-60, alpha111-126, alpha146-162 and alpha182-198 gave T cells that responded in vitro to the correlate immunizing peptide. Only the T cells against the latter three peptides cross-reacted with tAChR. Peptide alpha146-162 exhibited the highest in vitro reaction with the immunizing peptide and cross-reaction with tAChR. T cells obtained by immunization of B6 with an equimolar mixture of the peptides responded in vitro to peptides alpha111-126, alpha146-162 and alpha182-198 and cross-reacted very strongly with tAChR. In bm12 and C3H/He, a number of peptides evoked, when used individually as immunogens, strong or moderate T cell responses that recognized in vitro the correlate immunizing peptide but cross-reacted poorly with tAChR. Immunization with the mixture of the peptides gave T cells that recognized several peptides in each strain butdid not cross-react with alpha146-162 or tAChR. The results indicate that the ability to recognize alpha146-162 or AChR by T cells against peptides resulting from receptor degradation can account for the susceptibility to, and aggravation of, MG in B6.

PMID: 9583739 [PubMed - indexed for MEDLINE]

1: Eur J Epidemiol. 1992 Jan;8(1):1-8.

Links

Characteristics of toxin-neutralization by anti-tetanus human monoclonal antibodies directed against the three functional domains [A], [B] and [C] of the tetanus toxin molecule and a reliable method for evaluating the protective effects of monoclonal antibodies.

Matsuda M, **Kamei M**, **Sugimoto N**, **Ma Y**, **Hashizume S**.

Department of Tuberculosis Research, Osaka University, Japan.

Five anti-tetanus human monoclonal antibodies (MAbs) produced by hybrid cell lines we established previously were characterized. Their abilities to neutralize tetanus toxin in vitro and to protect mice against challenge with toxin were studied by observing the changes in the progress of symptoms in mice. Immunostaining showed that MAbs MAb-G4 and G2 recognized the N-terminal domain, [A] and the C-terminal domain, [C] of the tetanus toxin molecule, respectively, while MAbs MAb-G1, G3 and G6 recognized its middle domain, [B]. Enzyme-linked immunosorbent assay showed that the binding affinity of MAb-G3 was 2.9 x 10(10) M-1 and those of the other MAbs were as high as approximately 10(11) M-1. In in vitro neutralization experiments, at sufficient doses all the MAbs as single reagents protected mice completely against the effect of tetanus toxin. However, at lower doses than those sufficient to rescue mice, the kinetic patterns of progress of symptoms with the individual MAbs differed with each other and, except for MAb-G4, were different from that of anti-tetanus human polyclonal antibody. They suppressed the development and/or slowed the rate of progress of symptoms for over 96 h and delayed death of the mice. We propose that the comparison of the minimum survival dose with that of human polyclonal antibody of known international units is a reliable method for estimating the actual protective activity of a MAb. Intravenous (IV) injection of doses of individual MAbs or their mixtures at over 0.03 IU per mouse protected mice from subsequent challenge with 20 MLD of tetanus toxin.(ABSTRACT TRUNCATED AT 250 WORDS)

PMID: 1572415 [PubMed - indexed for MEDLINE]

1: Eur J Epidemiol. 1992 Jan;8(1):1-8.

Links

Characteristics of toxin-neutralization by anti-tetanus human monoclonal antibodies directed against the three functional domains [A], [B] and [C] of the tetanus toxin molecule and a reliable method for evaluating the protective effects of monoclonal antibodies.

Matsuda M, Kamei M, Sugimoto N, Ma Y, Hashizume S.

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Five anti-tetanus human monoclonal antibodies (MAbs) produced by hybrid cell lines we established previously were characterized. Their abilities to neutralize tetanus toxin in vitro and to protect mice against challenge with toxin were studied by observing the changes in the progress of symptoms in mice. Immunostaining showed that MAbs MAb-G4 and G2 recognized the N-terminal domain, [A] and the C-terminal domain, [C] of the tetanus toxin molecule, respectively, while MAbs MAb-G1, G3 and G6 recognized its middle domain, [B]. Enzyme-linked immunosorbent assay showed that the binding affinity of MAb-G3 was 2.9 x 10(10) M-1 and those of the other MAbs were as high as approximately 10(11) M-1. In in vitro neutralization experiments, at sufficient doses all the MAbs as single reagents protected mice completely against the effect of tetanus toxin. However, at lower doses than those sufficient to rescue mice, the kinetic patterns of progress of symptoms with the individual MAbs differed with each other and, except for MAb-G4, were different from that of anti-tetanus human polyclonal antibody. They suppressed the development and/or slowed the rate of progress of symptoms for over 96 h and delayed death of the mice. We propose that the comparison of the minimum survival dose with that of human polyclonal antibody of known international units is a reliable method for estimating the actual protective activity of a MAb. Intravenous (IV) injection of doses of individual MAbs or their mixtures at over 0.03 IU per mouse protected mice from subsequent challenge with 20 MLD of tetanus toxin.(ABSTRACT TRUNCATED AT 250 WORDS)

PMID: 1572415 [PubMed - indexed for MEDLINE]

1: Hybridoma. 1986 Spring;5(1):21-31.

Links

Protection of mice against tetanus toxin by combination of two human monoclonal antibodies recognizing distinct epitopes on the toxin molecule.

Ziegler-Heitbrock HW, **Reiter C**, **Trenkmann J**, **Futterer A**, **Riethmuller G**.

Human B-lymphocytes were fused with the human lymphoblastoid B-cell line WI-L2-729 HF2. Hybridoma frequencies were in the range of 10(-5) when the mononuclear cells were (a) prestimulated with pokeweed mitogen (PWM), (b) fused with polyethyleneglycol (PEG), and (c) selected in a hypoxanthine-azaserine (HAza) containing medium. To generate monoclonal antibodies (MAb) specific for tetanus toxin (TToxin) human spleen cells were precultured with PWM plus tetanus toxoid (TToxoid) in two separate fusions. Two hybridomas were selected based on high binding activity using an enzyme-linked immunosorbent assay (ELISA) for TToxoid. Both hybridomas, cloned twice and designated anti-TT1 and anti-TT2, exhibited a near tetraploid karyotype and showed stable production of antibody (0.15 micrograms/ml) over several months. Using ELISA for fragments of TToxin and the immunoblotting technique, the two IgG1 monoclonal antibodies were found to bind to the heavy chain portion of the B-fragment (anti-TT1) and on the C-fragment (anti-TT2) of the toxin. When tested in an ELISA with TToxin the combination of anti-TT1 and anti-TT2 showed higher binding activity than either reagent alone. In an in vivo neutralization assay mice were completely protected against TToxin by the combination of the two antibodies while either antibody alone resulted only in a delay of death of the mice. These findings demonstrate that a cocktail of appropriate human monoclonal antibodies can be far superior to a single reagent when used in a therapeutic setting.

PMID: 2420699 [PubMed - indexed for MEDLINE]

1: Mol Immunol. 1994 Oct;31(15):1141-8.

Links

Synthetic peptide antigens of tetanus toxin.

Fischer PM, **Howden ME**.

Department of Biological Sciences, Deakin University, Geelong, Victoria, Australia.

In this study the immunochemical structure of the heavy chain polypeptide from tetanus toxin was studied. Numerous antigenic determinants were identified by probing a set of overlapping peptides derived from the amino acid sequence of tetanus toxin with polyclonal anti-toxoid antibody preparations. Synthetic antigens representing continuous epitopes were prepared and used to immunize mice. The capacity of the resulting anti-peptide antibodies to react with tetanus toxin in vitro and in vivo was determined. The majority of antibodies bound to tetanus toxin and three epitopes capable of eliciting neutralizing antibodies were identified.

Mavoungou E, Toure FS, Yaba P, Delicat A, Poaty-Mavoungou V. Related Articles, Links

Peptide immunization restimulates the memory CD4 T cell response but fails to induce cytotoxic T lymphocytes in cynomolgus monkeys.

J Med Primatol. 1998 Aug;27(4):202-9.
PMID: 9879861 [PubMed - indexed for MEDLINE]

1: Chin Med J (Engl). 1999 Aug;112(8):691-7.

Links

A recombinant multi-epitope, multi-stage malaria vaccine candidate expressed in Escherichia coli.

Li M, Bi H, Dong W, Xu W, Li Q, Li Y.

Institute of Tropical Medicine, First Military Medical University, Guangzhou, 510515, China.

OBJECTIVE: To construct and evaluate a recombinant multi-epitope, multistage malaria vaccine candidate expressed in Escherichia coli (E. coli). METHODS: A hybrid gene (HGF) encoding several putative immunodominant T or T/B epitopes from MSP-1, MSP-2, Pf155/RESA of Plasmodium falciparum (P. falciparum) and two immune-stimulating epitopes from interleukin-1 and tetanus toxin was synthesized. Two copies of HGF and a copy of gene encoding Pattaroyo's Spf66 were connected together to construct a sandwich hybrid gene HGFSP. The gene was cloned into an expression vector pWR450-I for production of a fusion protein with beta-galactosidase. Efficacy of this vaccine candidate in inducing specific immunity against malaria parasites was evaluated. RESULTS: Immunization of different species of animals with purified recombinant peptide showed that the peptide was able to induce remarkable antibody response to the immunized peptide as well as falciparum malaria parasites. The epitopes included in the construct could induce antibodies against the intact parasite proteins as demonstrated by western blotting, indicating the epitopes retained their antigenicity in the new peptide construct. Antibodies from animals immunized with recombinant HGFSP peptide exhibited good ability in inhibition of the in vitro growth of malaria parasites, augmentation of phagocytosis of the parasites or infected RBC by phagocytes, and facilitation of antibody dependent cell mediated cytotoxicity to the cultured malaria parasites. CONCLUSION: The recombinant peptide seems to be a potential candidate which is valuable for further investigation.

PMID: 11601273 [PubMed - indexed for MEDLINE]

: Mol Immunol. 1994 Oct;31(15):1141-8.

Links

Synthetic peptide antigens of tetanus toxin.

Fischer PM, Howden ME.

Department of Biological Sciences, Deakin University, Geelong, Victoria, Australia.

In this study the immunochemical structure of the heavy chain polypeptide from tetanus toxin was studied. Numerous antigenic determinants were identified by probing a set of overlapping peptides derived from the amino acid sequence of tetanus toxin with polyclonal anti-toxoid antibody preparations. Synthetic antigens representing continuous epitopes were prepared and used to immunize mice. The capacity of the resulting anti-peptide antibodies to react with tetanus toxin in vitro and in vivo was determined. The majority of antibodies bound to tetanus toxin and three epitopes capable of eliciting neutralizing antibodies were identified.

PMID: 7935502 [PubMed - indexed for MEDLINE]

J Infect Dis. 1997 Feb;175(2):382-91.

Links

Epitope repertoire of human CD4+ T cells on tetanus toxin: identification of immunodominant sequence segments.

Diethelm-Okita BM, **Raju R**, **Okita DK**, **Conti-Fine BM**.

Department of Biochemistry, College of Biological Sciences, University of Minnesota, St. Paul 55108, USA.

Sequence regions of tetanus toxin-forming CD4+ cell epitopes in 8 HLA-disparate subjects were identified. Overlapping synthetic peptides corresponding to the complete tetanus toxin sequence were used to test, in a proliferation assay, unselected blood CD4+ cells or CD4+ cell lines propagated by stimulation with tetanus toxoid. The CD4+ cell lines recognized most peptides recognized by the blood CD4+ cells and they recognized additional peptides. Their responses were stronger than those of unselected blood CD4+ cells. Two peptides were recognized by all subjects: one largely overlapped a tetanus toxin sequence region previously identified as a "universal" T cell epitope. Thirteen other peptides elicited a CD4+ cell response in 6 or 7 of the 8 subjects, and another 10 elicited responses in 5 subjects.

PMID: 9203659 [PubMed - indexed for MEDLINE]

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CLUSTAL W (1.82) multiple sequence alignment


sp|P04958|TETX_CLOTE          -PITINNFRYSDPVNNDTIIMMEPPYCKGLDIYYKAFKITDRIWIVPERY
tr|Q7B8V4|Q7B8V4_CLOBO        MPFVNKQFNYKDPVNGVDIAYIKIPNAG-QMQPVKAFKIHNKIWVIPERD
sp|P10845|BXA1_CLOBO          -PFVNKQFNYKDPVNGVDIAYIKIPNVG-QMQPVKAFKIHNKIWVIPERD
tr|Q2PPK6|Q2PPK6_CLOBO        MPFVNKQFNYKDPVNGVDIAYIKIPNAG-QMQPVKAFKIHNKIWVIPERD
sp|Q45894|BXA2_CLOBO          -PFVNKQFNYKDPVNGVDIAYIKIPNAG-QMQPVKAFKIHNKIWVIPERD
tr|Q3LRX8|Q3LRX8_CLOBO        MPLVNQQINYYDPVNGVDIAYIKIPNAG-KMQPVKAFKIHNKVWVIPERD
                               *:. :::.* ****.  *  :: *         ***** :::*::***

sp|P04958|TETX_CLOTE          EFGTKPE-DFNPPSSLIEGASEYYDPNYLRTDSDKDRFLQTMVKLFNRIK
tr|Q7B8V4|Q7B8V4_CLOBO        TFTNPEEGDLNPPPEAKQVPVSYYDSTYLSTDNEKDNYLKGVTKLFERIY
sp|P10845|BXA1_CLOBO          TFTNPEEGDLNPPPEAKQVPVSYYDSTYLSTDNEKDNYLKGVTKLFERIY
tr|Q2PPK6|Q2PPK6_CLOBO        TFTNPEEGDLNPPPEAKQVPVSYYDSTYLSTDNEKDNYLKGVTKLFERIY
sp|Q45894|BXA2_CLOBO          TFTNPEEGDLNPPPEAKQVPVSYYDSTYLSTDNEKDNYLKGVTKLFERIY
tr|Q3LRX8|Q3LRX8_CLOBO        IFTNPEEVDLNPPPEAKQVPISYYDSAYLSTDNEKDNYLKGVIKLFERIY
                               * .  * *:***..  : . .***. ** **.:**.:*: : ***:**

sp|P04958|TETX_CLOTE          NNVAGEALLDKIINAIPYLGNSYSLLDKFDTNSNSVSFNLLEQDPSGATT
tr|Q7B8V4|Q7B8V4_CLOBO        STDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDG------S
sp|P10845|BXA1_CLOBO          STDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDG------S
tr|Q2PPK6|Q2PPK6_CLOBO        STDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDG------S
sp|Q45894|BXA2_CLOBO          STDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDG------S
tr|Q3LRX8|Q3LRX8_CLOBO        STDLGRMLLISIVRGIPFWGGGKIDTELKVIDTNCINIIQLDD------S
                               ..  *. ** .*:..**: *..    :    ::*.:..   :       :

sp|P04958|TETX_CLOTE          KSAMLTNLIIFGPGPVLNKNEVRGIVLRVDNKNYFPCRDGFGSIMQMAFC
tr|Q7B8V4|Q7B8V4_CLOBO        YRSEELNLVIIGPSADIIQFECKSFGHEVLN----LTRNGYGSTQYIRFS
sp|P10845|BXA1_CLOBO          YRSEELNLVIIGPSADIIQFECKSFGHEVLN----LTRNGYGSTQYIRFS
tr|Q2PPK6|Q2PPK6_CLOBO        YRSEELNLVIIGPSADIIQFECKSFGHDVLN----LTRNGYGSTQYIRFS
sp|Q45894|BXA2_CLOBO          YRSEELNLVIIGPSADIIQFECKSFGHDVLN----LTRNGYGSTQYIRFS
tr|Q3LRX8|Q3LRX8_CLOBO        YRSEELNLAIIGPSANIIESQCSSFRDDVLN----LTRNGYGSTQYIRFS
                                 :   ** *:**.. : : :  .:   * *      *:*:**   : *.

sp|P04958|TETX_CLOTE          PEYVPTFDNVIENITSLTIGKSKYFQDPALLLMHELIHVLHGLYGMQVSS
tr|Q7B8V4|Q7B8V4_CLOBO        PDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHELIHAGHRLYGIAINP
sp|P10845|BXA1_CLOBO          PDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHELIHAGHRLYGIAINP
tr|Q2PPK6|Q2PPK6_CLOBO        PDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHELIHAEHRLYGIAINP
sp|Q45894|BXA2_CLOBO          PDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHELIHAEHRLYGIAINP
tr|Q3LRX8|Q3LRX8_CLOBO        PDFTVGFEESLEVDTNPLLGAGKFAQDPAVALAHELIHAEHRLYGIAINT
                               *::.  *:: :*  *.  :* .*:  ***: * *****. * ***: :..

sp|P04958|TETX_CLOTE          HEIIPSKQEIYMQHT-YPISAEELFTFGGQDANLISIDIKNDLYEKTLND
tr|Q7B8V4|Q7B8V4_CLOBO        NRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNK
sp|P10845|BXA1_CLOBO          NRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNK
tr|Q2PPK6|Q2PPK6_CLOBO        NRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNK
sp|Q45894|BXA2_CLOBO          NRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNK
tr|Q3LRX8|Q3LRX8_CLOBO        NRVFKVNTNAYYEMAGLEVSLEELITFGGNDAKFIDSLQKKEFSLYYYNK
                               :.::  : : * : :   :* *** ****:**::*.   ::::     *.

sp|P04958|TETX_CLOTE          YKAIANKLSQVTSCNDPNIDIDSYKQIYQQKYQFDKDSNGQYIVNEDKFQ
tr|Q7B8V4|Q7B8V4_CLOBO        FKDIASTLNKAKSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFD
sp|P10845|BXA1_CLOBO          FKDIASTLNKAKSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFD
tr|Q2PPK6|Q2PPK6_CLOBO        FKDVASTLNKAKSIIGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFD
sp|Q45894|BXA2_CLOBO          FKDVASTLNKAKSIIGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFD
tr|Q3LRX8|Q3LRX8_CLOBO        FKDIASTLNKAKSIVGTTASLQYMKNVFKEKYLLSEDATGKFLVDRLKFD
                               :* :*..*.:..*  ... .::  *::::::** :.:*:.*:: *:. **:

sp|P04958|TETX_CLOTE          ILYNSIMYGFTEIELGKKFNIKTRLSYFSMNHDPVKIPNLLDDTIYNDTE
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tr|Q7B8V4|Q7B8V4_CLOBO      KLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKIN-IVPKVNYTIYD
sp|P10845|BXA1_CLOBO        KLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKIN-IVPKVNYTIYD
tr|Q2PPK6|Q2PPK6_CLOBO      KLYKMLTEIYTEDNFVNFFKVINRKTYLNFDKAVFRIN-IVPDENYTIKD
sp|Q45894|BXA2_CLOBO        KLYKMLTEIYTEDNFVNFFKVINRKTYLNFDKAVFRIN-IVPDENYTIKD
tr|Q3LRX8|Q3LRX8_CLOBO      ELYKLLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKIN-IVPDVNYTIHD
                             **: :   :** :: : *:: .* :*:.:::  .:*  :: .  *.  :

sp|P04958|TETX_CLOTE        GFNIESKDLKSEYKGQNMRVNTNAFRNVD-GSGLVSKLIGLCKKIIPPTN
tr|Q7B8V4|Q7B8V4_CLOBO      GFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSK
sp|P10845|BXA1_CLOBO        GFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSK
tr|Q2PPK6|Q2PPK6_CLOBO      GFNLKGANLSINFNGQNTEINSRNFTRLKNFTGLFEFYKLLCVRGIIPFK
sp|Q45894|BXA2_CLOBO        GFNLKGANLSTNFNGQNTEINSRNFTRLKNFTGLFEFYKLLCVRGIIPFK
tr|Q3LRX8|Q3LRX8_CLOBO      GFNLRNTNLAANFNGQNIEINNKNFDKLKNFTGLFEFYKLLCVRGIITSK
                            ***:.. :*  :::*** .:*.  * .:.  :**..    ** : * . :

sp|P04958|TETX_CLOTE        IRENLYNRTASLTDLGGELCIKIKNEDLTFIAEKNSFSEEPFQDEIVSYN
tr|Q7B8V4|Q7B8V4_CLOBO      TKSLDKGYNKALN----DLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSD
sp|P10845|BXA1_CLOBO        TKSLDKGYNKALN----DLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSD
tr|Q2PPK6|Q2PPK6_CLOBO      TKSLDEGYNKALN----DLCIKVNNWDLFFSPSEDNFTNDLDKVEEITAD
sp|Q45894|BXA2_CLOBO        TKSLDEGYNKALN----DLCIKVNNWDLFFSPSEDNFTNDLDKVEEITAD
tr|Q3LRX8|Q3LRX8_CLOBO      TKSLDEGYNKALN----ELCIKVNNWDLFFSPSEDNFTNDLDKVEEITSD
                             :.   . . :*.    :****::* ** * ..::.*:::  : * :: :

sp|P04958|TETX_CLOTE        TKNKPLNFNYSLDKIIVDYNLQSKITLPNDRTTP---VTKGIPYAPEYKS
tr|Q7B8V4|Q7B8V4_CLOBO      TNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELMPNI
sp|P10845|BXA1_CLOBO        TNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELMPNI
tr|Q2PPK6|Q2PPK6_CLOBO      TNIEAAEENISLDLIQQYYLTFDFDNEPENISIENLSSDIIGQLEPMPNI
sp|Q45894|BXA2_CLOBO        TNIEAAEENISLDLIQQYYLTFDFDNEPENISIENLSSDIIGQLEPMPNI
tr|Q3LRX8|Q3LRX8_CLOBO      TNIEAAEENISLDLIQQYYLNFNFDNEPENTSIENLSSDIIGQLEPMPNI
                            *: :. : * *** *   *   .  . *:: :                 :

sp|P04958|TETX_CLOTE        NAASTIEIHNIDDNTIYQYLYAQKSPTTLQRITMTNSVDDALINSTKIYS
tr|Q7B8V4|Q7B8V4_CLOBO      ERFPNGKKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYT
sp|P10845|BXA1_CLOBO        ERFPNGKKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYT
tr|Q2PPK6|Q2PPK6_CLOBO      ERFPNGKKYELDKYTMFHYLRAQEFEHGDSRIILTNSAEEALLKPNVAYT
sp|Q45894|BXA2_CLOBO        ERFPNGKKYELDKYTMFHYLRAQEFEHGDSRIILTNSAEEALLKPNVAYT
tr|Q3LRX8|Q3LRX8_CLOBO      ERFPNGKKYELNKYTMFHYLRAQEFKHSNSRIILTNSAKEALLKPNIVYT
                            :  .. : ::::. *:::** **:     .** :***..:**::..  *:

sp|P04958|TETX_CLOTE        YFPS-VISKVNQGAQGILFLQWVRDIIDDFTNESSQKTTIDKISDVSTIV
tr|Q7B8V4|Q7B8V4_CLOBO      FFSSDYVKKVNKATEAAMFLGWVEQLVYDFTDETSEVSTTDKIADITIII
sp|P10845|BXA1_CLOBO        FFSSDYVKKVNKATEAAMFLGWVEQLVYDFTDETSEVSTTDKIADITIII
tr|Q2PPK6|Q2PPK6_CLOBO      FFSSKYVKKINKAVEAFMFLNWAEELVYDFTDETNEVTTMDKIADITIIV
sp|Q45894|BXA2_CLOBO        FFSSKYVKKINKAVEAFMFLNWAEELVYDFTDETNEVTTMDKIADITIIV
tr|Q3LRX8|Q3LRX8_CLOBO      FFSSKYIKAINKAVEAVTFVNWIENLVYDFTDETNEVSTMDKIADITIVI
                            :*.*  :. :*:..:.  *: * .::: ***:*:.: :* ***:*:: ::

sp|P04958|TETX_CLOTE        PYIGPALNIVKQGYEGNFIGALETTGVVLLLEYIPEITLPVIAALSIAES
tr|Q7B8V4|Q7B8V4_CLOBO      PYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALVSY
sp|P10845|BXA1_CLOBO        PYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALVSY
tr|Q2PPK6|Q2PPK6_CLOBO      PYIGPALNIGNMLSKGEFVEAIIFTGVVAMLEFIPEYALPVFGTFAIVSY
sp|Q45894|BXA2_CLOBO        PYIGPALNIGNMLSKGEFVEAIIFTGVVAMLEFIPEYALPVFGTFAIVSY
tr|Q3LRX8|Q3LRX8_CLOBO      PYIGPALNIGNMIYKGEFVEAIIFSGAVILLEIVPEIALPVLGTFALVSY
                            ********* :   :.:*: *:  :*.* :** :** ::**:.::::..

sp|P04958|TETX_CLOTE        STQKEKIIKTIDNFLEKRYEKWIEVYKLVKAKWLGTVNTQFQKRSYQMYR
tr|Q7B8V4|Q7B8V4_CLOBO      IANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKE
sp|P10845|BXA1_CLOBO        IANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKE
tr|Q2PPK6|Q2PPK6_CLOBO      IANKVLTVQTINNALSKRNEKWDEVYKYTVTNWLAKVNTQIDLIREKMKK
sp|Q45894|BXA2_CLOBO        IANKVLTVQTINNALSKRNEKWDEVYKYTVTNWLAKVNTQIDLIREKMKK
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tr|Q3LRX8|Q3LRX8_CLOBO      VSNKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAIVNTQINLIREKMKK
                             ::*   ::**:* *.** *** ****   ::**. ****::    :* .

sp|P04958|TETX_CLOTE        SLEYQVDAIKKIIDYEYKIYSGPDKEQIADEINNLKNKLEEKANKAMINI
tr|Q7B8V4|Q7B8V4_CLOBO      ALENQAEATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINKAMINI
sp|P10845|BXA1_CLOBO        ALENQAEATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINKAMINI
tr|Q2PPK6|Q2PPK6_CLOBO      ALENQAEATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINSAMINI
sp|Q45894|BXA2_CLOBO        ALENQAEATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINSAMINI
tr|Q3LRX8|Q3LRX8_CLOBO      ALENQAEATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINSAMINI
                            :** *.:* * **:*:*: *:  :*::*  :*::*..**:*. *.*****

sp|P04958|TETX_CLOTE        NIFMRESSRSFLVNQMINEAKKQLLEFDTQSKNILMQYIKANSKFIGITE
tr|Q7B8V4|Q7B8V4_CLOBO      NKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYDN-RGTLIGQ
sp|P10845|BXA1_CLOBO        NKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYDN-RGTLIGQ
tr|Q2PPK6|Q2PPK6_CLOBO      NKFLDQCSVSYLMNSMIPYAVKRLKDFDASVRDVLLKYIYDN-RGTLVLQ
sp|Q45894|BXA2_CLOBO        NKFLDQCSVSYLMNSMIPYAVKRLKDFDASVRDVLLKYIYDN-RGTLVLQ
tr|Q3LRX8|Q3LRX8_CLOBO      NKFLDQCSVSYLMNSMIPYAVKRLKDFDASVRDVLLKYIYDN-RGTLIGQ
                            * *: :.* *:*:*.**  . *:* :**:. :: *::**  * :   : :

sp|P04958|TETX_CLOTE        LKKLESKINKVFSTPIPFSYSKNLD--CWVDNEEDIDVILKKSTILNLDI
tr|Q7B8V4|Q7B8V4_CLOBO      VDRLKDKVNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNIINTSILNLRY
sp|P10845|BXA1_CLOBO        VDRLKDKVNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNIINTSILNLRY
tr|Q2PPK6|Q2PPK6_CLOBO      VDRLKDEVNNTLSADIPFQLSKYVDNKKLLSTFTEYIKNIVNTSILSIVY
sp|Q45894|BXA2_CLOBO        VDRLKDEVNNTLSADIPFQLSKYVDNKKLLSTFTEYIKNIVNTSILSIVY
tr|Q3LRX8|Q3LRX8_CLOBO      VNRLKDKVNNTLSADIPFQLSKYVDNKKLLSTFTEYIKNITNASILSIVY
                            :.:*:.::*:.:*: ***. ** :*    :..  :    : :::**.:

sp|P04958|TETX_CLOTE        NNDIISDISGFNSSVITYPDAQLVPGINGKAIHLVNNESSEVIVHKAMDI
tr|Q7B8V4|Q7B8V4_CLOBO      ESNHLIDLSRYASKINIGSKVNFDP-IDKNQIQLFNLESSKIEVILKNAI
sp|P10845|BXA1_CLOBO        ESNHLIDLSRYASKINIGSKVNFDP-IDKNQIQLFNLESSKIEVILKNAI
tr|Q2PPK6|Q2PPK6_CLOBO      KKDDLIDLSRYGAKINIGDRVYYDS-IDKNQIKLINLESSTIEVILKNAI
sp|Q45894|BXA2_CLOBO        KKDDLIDLSRYGAKINIGDRVYYDS-IDKNQIKLINLESSTIEVILKNAI
tr|Q3LRX8|Q3LRX8_CLOBO      KDDDLIDLSRYGAEIYNGDKVYYNS-IDKNQIRLINLESSTIEVILKKAI
                            :.: : *:* : :.:     .   . *: : *:*.* *** : *     *

sp|P04958|TETX_CLOTE        EYNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISSMKKHSLSIGSGW
tr|Q7B8V4|Q7B8V4_CLOBO      VYNSMYENFSTSFWIRIPKYFN---SISLNNEYTIINCMENN-----SGW
sp|P10845|BXA1_CLOBO        VYNSMYENFSTSFWIRIPKYFN---SISLNNEYTIINCMENN-----SGW
tr|Q2PPK6|Q2PPK6_CLOBO      VYNSMYENFSTSFWIKIPKYFS---KINLNNEYTIINCIENN-----SGW
sp|Q45894|BXA2_CLOBO        VYNSMYENFSTSFWIKIPKYFS---KINLNNEYTIINCIENN-----SGW
tr|Q3LRX8|Q3LRX8_CLOBO      VYNSMYENFSTSFWIRIPKYFN---SISLNNEYTIINCMENN-----SGW
                             **.*::**:.***:::**      .   .***:**..::::     ***

sp|P04958|TETX_CLOTE        SVSLKGNNLIWTLKDSAGEVRQITFRDLPDKFNAYLANKWVFITITNDRL
tr|Q7B8V4|Q7B8V4_CLOBO      KVSLNYGEIIWTLQDTQEIKQRVVFKYSQMINISDYINRWIFVTITNNRL
sp|P10845|BXA1_CLOBO        KVSLNYGEIIWTLQDTQEIKQRVVFKYSQMINISDYINRWIFVTITNNRL
tr|Q2PPK6|Q2PPK6_CLOBO      KVSLNYGEIIWTLQDNKQNIQRVVFKYSQMVNISDYINRWIFVTITNNRL
sp|Q45894|BXA2_CLOBO        KVSLNYGEIIWTLQDNKQNIQRVVFKYSQMVNISDYINRWIFVTITNNRL
tr|Q3LRX8|Q3LRX8_CLOBO      KVSLNYGEIIWTFQDTQEIKQRVVFKYSQMINISDYINRWIFVTITNNRI
                            .***: .::***::*.    :::.*:       :   *:*:*:****:*:

sp|P04958|TETX_CLOTE        SSANLYINGVLMGSAEITGLGAIREDNNITLKLDRCNNNNQYVSIDKFRI
tr|Q7B8V4|Q7B8V4_CLOBO      NNSKIYINGRLIDQKPISNLGNIHASNNIMFKLDGCRDTHRYIWIKYFNL
sp|P10845|BXA1_CLOBO        NNSKIYINGRLIDQKPISNLGNIHASNNIMFKLDGCRDTHRYIWIKYFNL
tr|Q2PPK6|Q2PPK6_CLOBO      TKSKIYINGRLIDQKPISNLGNIHASNKIMFKLDGCRDPRRYIMIKYFNL
sp|Q45894|BXA2_CLOBO        TKSKIYINGRLIDQKPISNLGNIHASNKIMFKLDGCRDPRRYIMIKYFNL
tr|Q3LRX8|Q3LRX8_CLOBO      TKSKIYINGRLIDQKPISNLGNIHASNKIMFKLDGCRDPHRYIVIKYFNL
                            ..:::**** *:..  *:.** *: .*:* :*** *.: .:*: *. *.:

sp|P04958|TETX_CLOTE        FCKALNPKEIEKLYTSYLSITFLRDFWGNPLRYDTEYYLIPVASSSK--D
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tr|Q7B8V4|Q7B8V4_CLOBO      FDKELNEKEIKDLYDNQSNSGILKDFWGDYLQYDKPYYMLNLYDPNKYVD
sp|P10845|BXA1_CLOBO        FDKELNEKEIKDLYDNQSNSGILKDFWGDYLQYDKPYYMLNLYDPNKYVD
tr|Q2PPK6|Q2PPK6_CLOBO      FDKELNEKEIKDLYDSQSNSGILKDFWGNYLQYDKPYYMLNLFDPNKYVD
sp|Q45894|BXA2_CLOBO        FDKELNEKEIKDLYDSQSNSGILKDFWGNYLQYDKPYYMLNLFDPNKYVD
tr|Q3LRX8|Q3LRX8_CLOBO      FDKELSEKEIKDLYDNQSNSGILKDFWGDYLQYDKSYYMLNLYDPNKYVD
                            * * *. ***:.** .  .  :*:****: *:**. **:: : ...*  *

sp|P04958|TETX_CLOTE        VQLKNITDYMYLTNAPSYTNGKLNIYYRRLYNGLKFIIKRYTPNNEIDSF
tr|Q7B8V4|Q7B8V4_CLOBO      VNNVGIRGYMYLKGPRGSVMTTNIYLNSSLYRGTKFIIKKYASGN-KDNI
sp|P10845|BXA1_CLOBO        VNNVGIRGYMYLKGPRGSVMTTNIYLNSSLYRGTKFIIKKYASGN-KDNI
tr|Q2PPK6|Q2PPK6_CLOBO      VNNIGIRGYMYLKGPRGSVVTTNIYLNSTLYEGTKFIIKKYASGN-EDNI
sp|Q45894|BXA2_CLOBO        VNNIGIRGYMYLKGPRGSVVTTNIYLNSTLYEGTKFIIKKYASGN-EDNI
tr|Q3LRX8|Q3LRX8_CLOBO      VNNVGIRGYMYLKGPRDNVMTTNIYLNSSLYMGTKFIIKKYASGN-KDNI
                            *:  .* .****... . .  .       ** * *****:*:..*  *.:

sp|P04958|TETX_CLOTE        VKSGDFIKLYVSYNNNEHIVGYPKDGNAFNNLDRILRVGYNAPGIPLYKK
tr|Q7B8V4|Q7B8V4_CLOBO      VRNNDRVYINVVVKNKEYRLATNASQAGVEKILSALEIPDVGN--LSQVV
sp|P10845|BXA1_CLOBO        VRNNDRVYINVVVKNKEYRLATNASQAGVEKILSALEIPDVGN--LSQVV
tr|Q2PPK6|Q2PPK6_CLOBO      VRNNDRVYINVVVKNKEYRLATNASQAGVEKILSALEIPDVGN--LSQVV
sp|Q45894|BXA2_CLOBO        VRNNDRVYINVVVKNKEYRLATNASQAGVEKILSALEIPDVGN--LSQVV
tr|Q3LRX8|Q3LRX8_CLOBO      VRNNDRVYINVVVKNKEYRLATNASQAGVEKILSALEIPDVGN--LSQVV
                            *:..* : : *  :*:*: :.   .  ..:::   *.:   .

sp|P04958|TETX_CLOTE        MEAVKLRDLKTYSVQLKLYDDKNASLGLVGTHNGQIGNDPNRDILIASNW
tr|Q7B8V4|Q7B8V4_CLOBO      VMKSKNDQGITNKCKMNLQDNNGNDIGFIGFHQFNN-----IAKLVASNW
sp|P10845|BXA1_CLOBO        VMKSKNDQGITNKCKMNLQDNNGNDIGFIGFHQFNN-----IAKLVASNW
tr|Q2PPK6|Q2PPK6_CLOBO      VMKSKDDQGIRNKCKMNLQDNNGNDIGLIGFHQFNN-----IAKLVASNW
sp|Q45894|BXA2_CLOBO        VMKSKDDQGIRNKCKMNLQDNNGNDIGFIGFHLYDN-----IAKLVASNW
tr|Q3LRX8|Q3LRX8_CLOBO      VMKSKNDQGITNKCKMNLQDNNGNDIGFIGFHQFNN-----IAKLVASNW
                            :   *  :    . :::* *::. .:*::* *  :         *:****

sp|P04958|TETX_CLOTE        YFNHLK--DKILGCDWYFVPTDEGWTND--
tr|Q7B8V4|Q7B8V4_CLOBO      YNRQIERSSRTLGCSWEFIPVDDGWGERPL
sp|P10845|BXA1_CLOBO        YNRQIERSSRTLGCSWEFIPVDDGWGERPL
tr|Q2PPK6|Q2PPK6_CLOBO      YNRQVGKASRTFGCSWEFIPVDDGWGESSL
sp|Q45894|BXA2_CLOBO        YNRQVGKASRTFGCSWEFIPVDDGWGESSL
tr|Q3LRX8|Q3LRX8_CLOBO      YNRQIERSSRTLGCSWEFIPVDDGWRERPL
                            * .::   .: :**.* *:*.*:** :
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